CHEMICAL STUDIES ON *STREPTOMYCES'* ANTIBIOTICS

BY

CARL PAUL SCHAFFNER

A. B., Columbia University, 1950

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

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Introduction

During the past decade, an ever-increasing amount of scientific effort has been directed towards the search for naturally-occurring antibiotic substances with the net result that numerous compounds have been discovered and isolated. However, the total number of antibiotics that have contributed therapeutically to modern medicine is still small since many of the isolated compounds have proven to be either toxic or unsuitable under physiological conditions.

In a program covering antibiotics of antibacterial and antifungal nature, it is our hope that useful substances, effective against pathogenic fungi, *Mycobacterium tuberculosis*, and other organisms for which suitable chemotherapeutic agents are still needed, may be found. In addition we hope that with further knowledge of the structure of antibiotics we may relate chemical structure to physiological action perhaps with the net result that therapeutic compounds may be synthesized which might prove more effective and less toxic.

Discussion of Results

An antibiotic designated Antibiotic 65-24 (1) was isolated in crystalline form from fermentation culture broths of an actinomycete organism. The isolation procedure for this antibiotic involved solvent extractions, Craig countercurrent distribution with the solvent system benzene-methanol-water (5:5:1) and silicic acid adsorption chromatography with methanol-chloroform development. The antibiotic crystallizes from methanol-chloroform as colorless prisms, melting at 222-224°.

Antibiotic 65-24 has been tentatively assigned the empirical formula $C_{27}H_{38}N_6O_{10}$ (calculated molecular weight, 606.0). Rast molecular weight studies indicated a molecular weight range of 550-650. The antibiotic is essentially a neutral compound. It is soluble in chloroform and pyridine; moder-

ately soluble in most other organic solvents; insoluble in *n*-hexane and water at any pH. The antibiotic is highly optically active, and it possesses characteristic ultraviolet absorption at 243 and 318 mμ. The infrared spectrum of Antibiotic 65-24 strongly suggests the presence of substituted amide and ester linkages.

Antibiotic 65-24 gives negative or inconclusive results with a large number of qualitative organic tests. Vigorous alkaline degradation of the antibiotic gives rise to volatile basic and acidic compounds plus a fluorescent yellow substance. Paper chromatographic analysis of the alkaline hydrolysate indicated the presence of a minimum of four ninhydrin-positive components. Vigorous acid hydrolysis of Antibiotic 65-24 also results in the complete breakdown of the molecule. Paper chromatographic analysis of the acid hydrolysate indicated the presence of a minimum of four ninhydrin-positive components.

Mild methanolic sodium hydroxide treatment of Antibiotic 65-24 gives rise to an inactive acid which is insoluble in dilute hydrochloric acid. The formation of this acid represents some minor change in the intact molecule since more vigorous chemical degradation apparently gives rise to the same degradation products obtained from the intact antibiotic. It appears that this acid possesses an empirical formula and molecular weight very similar to that of the antibiotic.

Antibiotic 65-24 is very active biologically against the Gram-positive microorganisms, inhibiting growth at levels of less than 1γ per ml.; against the Gram-negative bacteria it is less effective. Preliminary toxicity studies on a crude preparation showed it to be very toxic to mice.

The isolation of three antifungal antibiotics was studied using partition chromatography. Crude preparations of Antibiotic 334 were best fractionated by a liquid-liquid phase partition chromatographic procedure (2). Silicic acid heavily equilibrated with strong phosphate buffer was used as the immobile phase while butanol-chloroform solvent systems were used as the mobile phase. Crude chloroform broth extracts of Antibiotics 74-36 and 285 were fractionated by a liquid-

solid phase partition method using silicic acid as adsorbent and methanol-chloroform as solvent systems.

The chemistry of streptothricin and its acidic degradation product Compound B (3) was further investigated. The periodate consumption by Compound B was studied at different pH levels. Apparently at pH 8.5 two moles of periodate are reduced in 30 minutes, three moles in 7 hours and a fourth mole after 24 hours. At pH 4.5 the first mole of periodate is reduced in 1 hour and the second mole only after 24 hours. At pH 2.0 Compound B reduces the first mole of periodate very slowly.

Compound B on reaction with the first mole of periodate at pH 4.5 yields one mole each of formaldehyde, ammonia, and a highly unstable compound which exhibits reducing properties.

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VITA

The author was born in Bayonne, New Jersey, on February 13, 1928. He attended elementary schools in that community and graduated from Bayonne High School in June, 1946. From September, 1946, until June, 1950, he attended Columbia University in New York City, receiving the degree of Bachelor of Arts from that institution. In September, 1950, he enrolled in the Graduate College of the University of Illinois. From the time of entrance to August, 1951, he held a Research Assistantship and from September, 1951, until June, 1953, he held a Research Fellowship jointly sponsored by the Upjohn Company, Eli Lilly and Company, and the Abbott Laboratories.